ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Radon Exposure, IL-6 Promoter Variants, and Lung Squamous Cell Carcinoma in Former Uranium Miners

Shuguang Leng, Cynthia L. Thomas, Amanda M. Snider, Maria A. Picchi, Wenshu Chen, Derall G. Willis, Teara G. Carr, Jacek Krzeminski, Dhimant Desai, Amin Shantu, Yong Lin, Marty R. Jacobson, and Steven A. Belinsky

http://dx.doi.org/10.1289/ehp.1409437

Received: 5 November 2014
Accepted: 11 September 2015
Advance Publication: 15 September 2015

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Radon Exposure, IL-6 Promoter Variants, and Lung Squamous Cell Carcinoma in Former Uranium Miners

Shuguang Leng,[1] Cynthia L. Thomas,[1] Amanda M. Snider,[1] Maria A. Picchi,[1] Wenshu Chen,[1] Derall G. Willis,[2] Teara G. Carr,[2] Jacek Krzeminski,[3] Dhimant Desai,[3] Amin Shantu,[3] Yong Lin,[1] Marty R. Jacobson,[2] and Steven A. Belinsky[1]

[1]Lung Cancer Program, Lovelace Respiratory Research Institute, Albuquerque, NM, USA; [2]Saccomanno Research Institute, St. Mary's Hospital & Medical Center, Grand Junction, CO, USA; [3]Department of Pharmacology, Penn State College of Medicine, Hershey, PA, USA

Address correspondence to Steven A. Belinsky, Lung Cancer Program, Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87108. Telephone: 505-348-9465. E-mail: sbelinsk@LRRI.org

Running title: IL-6 SNPs and lung cancer in uranium miners

Acknowledgments: We thank Drs Yushi Liu and Guodong Wu for the statistical assistance related to the latency analysis and SNP–IL-6 expression analysis using Merlin, respectively, Ms. Donna M. Klinge for sequencing and cloning IL-6 promoter, and Mr. Thomas J. Gagliano for the scientific editing of figures. This study was supported by NIEHS R01 ES015262, U.S. Department of Energy (DE-FG02-09ER64783), and NIH/NCI P30 CA118100. Dr. Chen was supported by a NCI award (R03CA167727).

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Disclaimers: The funding organizations played no role in the design and conduct of the study; in collection, management, analysis, and interpretation of the data; or in the presentation, review, or approval of the manuscript.

Competing financial interests: The authors declare they have no actual or potential competing financial interests.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Abstract

Background: High radon exposure is a risk factor for squamous cell carcinoma, a major lung cancer histology seen in former uranium miners. Radon exposure can cause oxidative stress leading to pulmonary inflammation. Interleukin-6 (IL-6) is a pro-carcinogenic inflammatory cytokine that plays a pivotal role in lung cancer development.

Objectives: We assessed whether single nucleotide polymorphisms (SNP) in the IL-6 promoter are associated with lung cancer in former uranium miners with high occupational exposure to radon gas.

Methods: Genetic associations were assessed in a case control study of former uranium miners (242 cases and 336 controls). A replication study was performed using data from the GENEVA Genome Wide Association Study of Lung Cancer and Smoking study. Functional relevance of the SNPs was characterized using *in vitro* approaches.

Results: Rs1800797 was associated with squamous cell carcinoma in miners, and with a shorter time between midpoint of the period of substantial exposure and diagnosis among the cases. Rs1800797 also was associated with lung cancer among never smokers in the GENEVA dataset. Functional studies identified that risk allele was associated with increased basal IL-6 mRNA level and greater promoter activity. Furthermore, fibroblasts with risk allele showed greater induction of IL-6 secretion by hydrogen peroxide or benzo(a)pyrene diolepoxide treatments.

Conclusions: An IL-6 promoter variant was associated with lung cancer in uranium miners and never smokers in two external study populations. The associations are strongly supported by the functional relevance that IL-6 promoter SNP affects basal expression and carcinogen-induced IL-6 secretion.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Introduction

Radon is an inert gas released during the decay of radium-226. Radon gas is ubiquitous in indoor and outdoor air and contaminates many underground mines (Sethi et al. 2012). Cohort studies of underground miners have established a strong association between high levels of radon exposure and increased risk for lung cancer (Archer 1988; Archer et al. 2004; Gilliland et al. 2000). Moreover, combined exposure to radon and tobacco smoke through uranium mining may further increase lung cancer risk (Archer 1988). Lung squamous cell carcinoma is the predominant histological type of lung cancer in former uranium miners that is likely driven by the localization of radon within the upper airways due to its binding to silica and diesel particles inhaled by the miners (Saccomanno et al. 1996; Samet 1989; Sethi et al. 2012). An association between residential radon exposure and lung cancer risk was also identified in seven North American case-control studies (Krewski et al. 2006).

The high-linear energy transfer alpha particles emitted by radon and radon daughters can directly attack genomic DNA to cause mainly DNA double strand breaks (Prise et al. 2001; Sethi et al. 2012). In addition, overproduction of reactive oxygen species in the lungs due to persistent radon exposure may cause oxidative stress leading to pulmonary inflammation, tissue damage, and eventually chronic lung diseases such as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and lung cancer (Archer et al. 1998; Iyer et al. 2000; Mapel et al. 1997; Narayanan et al. 1997; Rosanna and Salvatore 2012; Schubauer-Berigan et al. 2009). Strong associations between pulmonary inflammation as manifested by COPD and/or chronic mucous hypersecretion and risk for subsequent lung cancer incidence support the premise that persistent inflammation is involved in the etiology of lung cancer (Brenner et al. 2012; Wilson et al. 2008). Among the cytokines and chemokines produced from persistent pulmonary inflammation,

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

interleukin-6 (IL-6) plays a pivotal role in promoting cancer development as shown in studies using in vitro and in vivo models of lung carcinogenesis(Chen et al. 2012; Dougan et al. 2011; Gao et al. 2007; Ochoa et al. 2011; Qi et al. 2014).

IL-6 has a promoter with several single nucleotide polymorphisms (SNPs) showing large differences in minor allele frequency (MAF) across major ethnic populations. Greater induction of IL-6 promoter activity by norepinephrine, lipopolysaccharide, and IL-1 in an *in vitro* plasmid construct carrying the 'G' allele of rs1800795 was identified (Cole et al. 2010; Fishman et al. 1998). Gel shift assays confirmed the exclusive binding of GATA1 to the sequence containing the rs1800795 'G' allele following norepinephrine induction (Cole et al. 2010). However, results from population studies assessing the association between rs1800795 and plasma levels of IL-6 as an indicator for systematic inflammation were inconsistent (Fishman et al. 1998; He et al. 2009; Ljungman et al. 2009a; Ljungman et al. 2009b; Sousa et al. 2012; Zakharyan et al. 2012).

The current study assessed the association between IL-6 promoter SNPs and squamous cell carcinoma in uranium miners. Generalization to populations with residential radon exposure was examined using the GENEVA Genome Wide Association Study of Lung Cancer and Smoking study. The functional relevance of significant variants was also assessed *in vitro* using multiple cell types.

Methods

Former uranium miners. A cumulative incidence case-control study was conducted in Saccomanno Uranium Miner cohort of male former uranium miners (n = 17,000) who worked underground at the Colorado plateau and participated in sputum cytology screening for lung cancer detection between 1957 and 2002 (Saccomanno et al. 1996). A pool of confirmed deceased former uranium miners that contained 360 squamous cell carcinoma cases and 810 life-

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

time lung cancer-free miners with data available for essential variables that included age and smoking history at sputum collection, working level month (WLM), age at death, and age at lung cancer diagnosis and survival after lung cancer diagnosis for cases were used in the present study. Squamous cell carcinoma cases were identified from the St. Mary's Hospital Cancer Registry and the St. Mary's Saccomanno Research Institute Cancer Research Database. Because 94% of miners (n=1100) were Caucasian, the study was restricted to this ethnic group to minimize the bias due to ancestry differences. The sputum specimens proximal to cancer diagnosis for cases or the last follow-up for controls were used for DNA isolation. Subjects for whom DNA samples could not be recovered from sputum cytology slides based on amplification of a 180 bp DNA fragment in the human *KRAS* gene were excluded from this present study. A total of 267 cases and 383 controls were included for the genotyping study. The miners selected as the controls have cumulative radon exposure at work expressed as WLM and age at death similar to other life-time lung cancer-free uranium miners who were born during 1904 – 1933 (WLM, 0.8 versus 0.7 kWLMs; age at death, 68.7 versus 68.5 yrs). No personal identifiers accompanied the transfer of material from the St. Mary's Saccomanno Research Institute to Lovelace Respiratory Research Institute. This present study was conducted under an Institutional Review Board approved protocol which was exempt from informed consent requirements based on the Department of Health and Human Services regulations under 45CFR46.102(f) which defines a human subject as a living individual.

Cumulative radon exposure. WLM, a time-integrated measure of radon progeny exposure from mine air was calculated for each individual as the product of the exposure time in working months (1 month = 170 h) and average working level to estimate the cumulative radon exposure for each miner. Working level was defined as the measure of the energy released by airborne

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

radon progeny that was measured based on counting the emissions of alpha particles in a representative volumes of air (National Research Council (U.S.). Committee on Health Effects of Exposure to Radon. et al. 1994). One working level equals any combination of radon progeny in 1 liter of air that results in the ultimate emission of 130,000 MeV of energy from alpha particles. The information used for WLM assessment was from the mining history obtained for each miner and the accumulated mine measurement data collected by NIOSH over the years (Archer et al. 2004; Lundin et al. 1971). WLM was calculated up to cancer diagnosis for squamous cell carcinoma cases and for the entire uranium mining occupation for the controls.

GENEVA GWAS of Lung Cancer and Smoking study. Lung cancer cases (n=2522) and controls (n=2725) drawn from the Environment and Genetics in Lung Cancer Etiology Study (EAGLE, 1816 cases and 1984 controls) and the Prostate, Lung, Colon and Ovary Study (PLCO, 706 cases and 741 controls) Cancer Screening Trial comprised the GENEVA GWAS of Lung Cancer and Smoking study (Landi et al. 2009) with genotype data obtained using Illumina HumanHap550v3.0 chips (dbGaP Study Accession number: phs000093.v2.p2, see Supplemental Material, Table S1). The EAGLE and PLCO studies enrolled subjects from general populations from the Lombardy region of Italy and 10 locations throughout the United States, respectively (see Supplemental Material for a detailed introduction of these two studies), thus participants in these studies would not be expected to have occupational exposure to radon or other radiation. Allele dosage for rs1800797 was extracted from imputation data that was obtained using the 1000 Genomes Project European populations as the reference (Genomes Project et al. 2012). This study was selected to assess whether the association seen for rs1800797 in miners can be generalized to populations with residential radon exposure because of the large sample size and available smoking history data. Stratification analysis by smoking status or tumor histology was

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

conducted because a combined analysis of seven large scale case-control studies conducted in North America identified a significant association between residential radon exposure and lung cancer risk in adenocarcinoma (Krewski et al. 2006). In addition, the role of residential radon exposure in the etiology of lung cancer may be more prominent in never smokers (Samet et al. 2009). The informed consent document signed by the PLCO study participants allows use of these data by investigators for discovery and hypothesis generation in the investigation of the genetic contributions to cancer and other adult diseases as well as development of novel analytical approaches for GWAS. Use of the EAGLE dataset is limited to scientific genetic research related to the etiology, molecular basis, and outcome of lung disease and smoking. Thus, IRB approval for use of these deidentified data in genetic association analysis in the present study was not required.

SNP selection and genotyping. All common SNPs (minor allele frequency >0.05) within IL-6 locus (chr7: 22721293 – 22776691, genome build 36, see Supplemental Material, Figure S1) were predicted for their functional potential using SNP Web Info (Xu and Taylor 2009). Four SNPs (rs12700386, rs2069827, rs1800797, and rs2069840) in promoters not in high linkage disequilibrium (LD) ($r^2 < 0.22$) were suggested to affect the binding of transcription factors as evidenced by the fact that MATCH predicted a transcriptional factor binding site with one allele but not with the other or the difference in the matrix similarity scores or core similarity scores between the two alleles was ≥0.2 (Kel et al. 2003), and thus were selected for genotyping using the Taqman genotyping assay. Rs1800797 'A' allele is in almost perfect LD with rs1800795 'C' allele ($r^2 = 0.97$). Subjects with missing genotype data for more than two SNPs (5 squamous cell carcinoma cases and 23 controls) were excluded from the present analysis. Thus, 242 cases and 336 controls were eventually included in the genetic association analysis.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Real time PCR. RNA was isolated from low passage (≤2) primary human bronchial epithelial cell (BEC) cultures (n = 85) and human skin fibroblast lines (n = 6) that were established from cells obtained by bronchoscopy from current or former smokers seen at pulmonary clinic (Leng et al. 2012) or obtained from infant foreskin at University of New Mexico Hospital, respectively. BEC cultures established from unaffected sites were used for this experiment for patients with subsequent lung cancer diagnosis. TaqMan real-time PCR was conducted to quantify IL-6 gene expression in cDNA using the delta threshold cycle method with β-actin as the endogenous control.

Re-sequencing of the IL-6 promoter. Twenty-one alleles of the IL-6 promoter (690 bps, chr7:22732707-22733396) that contained nine rs1800797 A alleles and twelve rs1800797 G alleles were successfully amplified using primers listed in supplemental material, Table S2 from 13 human BEC cultures that were heterozygotes of rs1800797 and directionally cloned into the pGL2-basic Luciferase Reporter Vector (Promega) upstream of the luciferase coding sequence using MluI and BglII cloning sites following manufacture instruction (Promega Corporation, 2015). Dual direction Sanger sequencing was conducted to confirm the haplotype alleles that contained four SNPs: rs1800797, rs1800796, rs36215814, and rs1800795.

Luciferase reporter assay. pGL2 constructs generated using the method described above that contain two common haplotype alleles (A-G-A8T12-C and G-G-A10T11-G) of the IL-6 promoter were sequence verified for transfection experiments. Transfection was conducted in a human embryonic kidney cell line (HEK293) and a human lung fibroblast line (HFL1) both obtained directly from the American Type Culture Collection using TransIT-2020 transfection reagent (Mirus Bio LLC) and in a normal human bronchial epithelial cell line immortalized by insertion of the telomerase catalytic subunit and cyclin-dependent kinase 4 (HBEC2) obtained

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

from Drs. Shay and Minna, Southwestern Medical Center, Dallas, TX using Neon electroporation system (Life Technologies). Cells were harvested 48 h post transfection and reporter activity was measured using the Dual Luciferase Assay System (Promega). Control experiments were conducted in the same way as described except the IL-6 promoter constructs were replaced with promoterless or SV40 promoter constructs. The constructs carrying two major haplotype alleles (A-G-A8T12-C and G-G-A10T11-G) in the IL-6 promoter generated 5 to 30 times higher reporter activity relative to the promoterless construct in the three cell lines tested (not shown), suggesting that IL-6 has a robust promoter.

Benzo(a)pyrene diolepoxide (BPDE) and hydrogen peroxide (*H_2O_2*) induced IL-6 secretion in fibroblast lines. Lung fibroblasts appear to be a major source of IL-6 in the microenvironment of normal lungs based on the findings of greater basal and tobacco carcinogen-induced secretion of IL-6 in human lung fibroblasts compared to lung epithelial cells (Chen et al. 2012). A dose-dependent induction of IL-6 was observed in lung fibroblasts treated with BPDE (Chen et al. 2012) and this response was replicated in skin fibroblasts (Figure 1). Thus, due to the lack of lung fibroblasts with varied genotypes, the effect of IL-6 variants on IL-6 induction by lung carcinogen treatment was assessed in skin fibroblasts that are wild homozygote (GG, n=3) and heterozygote (AG, n=3) for rs1800797. Low passage skin fibroblast lines (n = 6) were maintained in DMEM with 10% fetal bovine serum (FBS). Prior to BPDE or H_2O_2 treatment, cells were washed twice with PBS to remove any residual FBS in the medium. Cells were then cultured in DMEM medium without FBS and treated with BPDE or H_2O_2 at varied concentrations for an hour. The medium with carcinogens was then replaced with fresh medium without FBS. The level of IL-6 in the medium was measured 24 h later using an enzyme-linked immunosorbent assay kit from eBioscience.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Statistical analysis. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between case-control status and each SNP using an additive inheritance model that evaluates the contribution of each allele for cancer risk. Models were adjusted for cumulative radon exposure [working level month (WLM)] which was modeled using one indicator variable for ⩾ 0.895 kWLM and one indicator variable for missing WLM (n = 27), with < 0.895 kWLM as the common reference exposure and for smoking packyears (⩾ 35 versus < 35) and age at sputum collection. The missing indicator variable for WLM was included to maximize the number of observations included in the analysis.

The complete record of the occupational history for underground uranium mining was available for 162 miners with squamous cell carcinoma diagnosis, thus allowing for calculation of the mid-induction latency defined as time from midpoint of the period of substantial exposure (an annual accumulative dose of ≥50.6 mSv) to squamous cell carcinoma diagnosis (Archer et al. 2004). All 162 miner cases achieved this level of exposure prior to the mid 1960's when the effective radiation control started to be implemented in underground uranium mines. The least square means of MIL for miners with GG, GA, and AA genotypes of rs1800797 were calculated using a generalized linear model with adjustment for underground mining history and smoking history surveyed at cancer diagnosis. The Cox proportional hazard models were used in 162 cases to estimate associations between IL-6 variants and the time to cancer diagnosis. Underground mining history (age at start of underground uranium mining, average WLM per month, and total years of underground mining) and smoking history (age at start of cigarette smoking, average number of cigarettes smoked per day, and total years of cigarette smoking, and number of years after quitting smoking) surveyed at squamous cell carcinoma diagnosis were included in the model for covariate adjustment.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

A generalized linear model was used to assess the association between rs1800797 and IL-6 expression in 85 primary human BECs and between subsequent lung cancer diagnosis and IL-6 expression in a subset of primary human BECs with adjustment for rs1800797 genotype coded as 0, 1, 2 for GG, GA, and AA (n = 78). Likehood ratio test implemented in Merlin was applied to test the association between rs1800797 and steady-state IL-6 expression (GI_10834983-S) in 79 EBV-transformed lymphoblastoid cell lines from CEU families in the HapMap project (Abecasis et al. 2002; Holm et al. 2010). The effect of rs1800797 and dose of carcinogen exposure on IL-6 secretion in human skin fibroblast lines (n = 6) were assessed using generalized linear models. The comparison of slopes for the dose-response curves by rs1800797 genotype was conducted using the likelihood ratio test by including an interaction term of genotype and carcinogen treatment in the generalized linear models. Two-sided p-value of 0.05 was used to define a significant association. Statistical analyses were conducted using SAS 9.2.

Results

Demographics of the miners. A total of 242 cases and 336 controls were eventually included in the genetic association analysis (Table 1). Cases were older and had higher packyears than controls at the time of the latest sputum collection. Half of the cases died within six months of diagnosis. Cases were an average of six years younger at death than controls. All study subjects were male and NHWs.

Association between IL-6 promoter SNPs and squamous cell carcinoma in miners. Miners with cumulative radon exposure levels ≥0.895 kWLM was a statistically significant risk factor for squamous cell carcinoma compared to miners with <0.895 kWLM (OR =1.51, 95%CI: 1.05, 2.18, p-Value =0.026 with adjustment for rs1800797 genotype, packyears, and age at sputum collection), consistent with radon progeny exposure as a risk factor for squamous cell carcinoma

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

in miners. More cases had missing WLM estimates than controls (OR =18.6, 95%CI: 4.2, 82.8, p-Value =0.00012). The variant allele 'A' of rs1800797 was a statistically significant risk factor for squamous cell carcinoma in former uranium miners (OR =1.36, 95%CI: 1.05, 1.75, p-Value =0.018, Table 2). Sensitivity analysis using miners with non-missing WLM estimates did not change the genetic associations observed for IL-6 promoter SNPs (OR =1.34, 95%CI: 1.04, 1.73, p-Value =0.023).

Association between IL-6 promoter SNPs and latency in miners with squamous cell carcinoma. The median of latency (19 y) in the 162 miners with squamous cell carcinoma was comparable to that seen for a large group of the American underground uranium miners (18.9 y, n =505) from who were either current smokers or quit smoking < 10 y when diagnosed with lung cancer (Archer et al. 2004). The least square means of MIL are 20.7 (standard error, 0.68), 19.5 (0.50), and 18.0 (0.73) for miners with GG, GA, and AA genotypes of rs1800797 calculated using a generalized linear model with adjustment for underground mining history and smoking history surveyed at cancer diagnosis (p-Value = 0.0075). Consistent with being a risk allele for squamous cell carcinoma, each copy of the 'A' allele of rs1800797 was associated with a hazard ratio (HR) of 1.57 (95%CI: 1.22, 2.01, p-Value =0.00037) for latency (Table 2).

Association between rs1800797 and risk for lung cancer in GENEVA dataset. The GWAS of Genetic association analysis in 2522 lung cancer cases and 2725 controls identified increased risk for lung cancer associated with rs1800797 'A' allele (OR=1.10, 95%CI: 1.01, 1.20, p-Value=0.04, Table 3). Associations between rs1800797 and lung cancer varied significantly by smoking status, with OR = 1.41 (95% CI: 1.05, 1.91) for each A allele in never smokers (interaction p-value = 0.05 for the difference from current smokers); OR = 1.08 (95% CI: 0.95, 1.24) for each A allele in former smokers (interaction p-value = 0.03 for the difference from

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

current smokers); and OR 1.06 (95% CI: 0.92, 1.22) in current smokers (Table 3). Associations with each rs1800797 A allele also varied by histologic subtype, with a significant positive association with adenocarcinoma (OR = 1.16; 1.04, 1.31; p-value = 0.009) compared with OR = 1.08 (95% CI: 0.93, 1.25; p-value 0.3) for squamous cell carcinoma (Table 3).

Association between rs1800797 and IL-6 expression. The risk allele of rs1800797 was associated with increased IL-6 expression in an allelic dose-dependent manner assessed in 85 primary human BECs from current and former smokers (p-value = 0.009) and 79 lymphoblastic cell lines from the HapMap CEU population (p-value = 0.034) (Table 4). Interestingly, human BECs collected from patients with subsequent lung cancer diagnosis (n = 56) had significantly higher IL-6 expression than those without (n = 22) (0.0029 ± 0.0034 vs. 0.0013 ± 0.0014, p-Value = 0.027 with adjustment for rs1800797 genotype).

Haplotype alleles in the IL-6 promoter. AGC and GGG are two major haplotype alleles in the IL-6 promoter that contain rs1800797, rs1800796, and rs1800795 and have cumulative allele frequency >0.95 in European populations from 1000 Genomes Project (Genomes Project et al. 2012). However, the phasing status is unclear between rs36215814 as an AnTn polymorphism located between rs1800796 and rs1800795 and other three SNPs. Cloned sequencing of 21 IL-6 promoter alleles amplified from 13 self-identified non-Hispanic white subjects that are heterozygotes for rs1800797 identified eight different compositions of the AnTn polymorphism with A8T12 enriched in AGC allele and A10T11 enriched in GGG allele (see Supplemental Material, Table S2). According to the allele frequencies for AGC (0.5) and GGG (0.45) in the European populations (Genomes Project et al. 2012), the estimated frequencies for haplotype alleles A-G-A8T12-C and G-G-A10T11-G are 0.5 and 0.15, respectively.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

IL-6 promoter activity by haplotype alleles. The reporter assay showed that haplotype allele A-G-A8T12-C carrying the risk allele of rs1800797 had 45-92% increased promoter activity compared to G-G-A10T11-G in HFL1, HEK293, and HBEC2 cell lines (p-Values < 6.2×10^{-4}, Figure 2).

IL-6 promoter SNP and the effect of H_2O_2 and BPDE on IL-6 secretion in human fibroblasts. Six skin fibroblast lines (three GGs and three AGs for rs1800797 as described in Methods) were used to assess whether the rs1800797 genotype could modify IL-6 secretion as the response to DNA damage indicative of radon or tobacco carcinogen exposures. IL-6 mRNA expression was 10 times higher in AG lines compared to GG lines (0.0021 ± 0.0011 versus 0.00020 ± 0.00009, P = 0.061, not shown). Treatment of cells with H_2O_2 and BPDE creates oxidative damage and BPDE-DNA adducts that mimic the damage induced by radon and the tobacco carcinogen benzo(a)pyrene, respectively (Narayanan et al. 1997; Tellez et al. 2011). The effect of rs1800797 genotype (coded as 0 for GG or 1 for AG) and dose of carcinogen exposure (concentrations in medium) on IL-6 secretion in human skin fibroblast lines (n = 6) were assessed using generalized linear models. A dose-dependent induction of IL-6 secretion was seen in skin fibroblasts treated with H_2O_2 and BPDE (p-Values < 0.0001, Figure 1). AG lines had consistently higher levels of IL-6 secretion than GG lines (p-Values < 0.015) with the different treatments. Moreover, the slopes for the induction of IL-6 secretion by increasing concentrations of H_2O_2 or BPDE was 74% and 39% greater in AG lines than GG lines (2.97 (0.44) versus 1.71 (0.20), p-Value = 0.017 for H_2O_2 and 0.24 (0.04) versus 0.17 (0.02), p-Value = 0.13 for BPDE), indicating a stronger induction kinetic for IL-6 secretion in AG lines. Furthermore, the strong correlation between IL-6 mRNA expression and protein secretion (Pearson correlation coefficient = 0.78) suggested that the induced IL-6 secretion by carcinogen treatment stems from gene transcription.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Discussion

This study comprehensively evaluated the association between four IL-6 promoter variants predicted to affect binding of transcription factors by *in silico* analyses and lung squamous cell carcinoma in former uranium miners with high levels of radon exposure. Significant associations were identified between rs1800797 and increased odds for squamous cell carcinoma and shortened latency for development of squamous cell carcinoma. Rs1800797 was associated with increased basal expression and induced secretion of IL-6 by fibroblasts in response to DNA damage *in vitro*, supporting a role for IL-6 in the association between rs1800797 and squamous cell carcinoma in the former uranium miners. The association between rs1800797 and squamous cell carcinoma among the former uranium miners was somewhat consistent with the association between rs1800797 and all lung cancers among never smokers in the GENEVA GWAS of Lung Cancer and Smoking study population, though the association was stronger for adenocarcinoma than squamous cell carcinoma when evaluated by case subtype. Radon exposures in GENEVA participants were not assessed, but radon is a potential cause of lung cancer among never smokers. This finding reinforces the importance of considering the levels of environmental exposures when studying the association between IL-6 promoter variants and environmental disease phenotype (Cole et al. 2010; Ljungman et al. 2009b). In addition, stratified analysis by tumor histology identified a significant association in lung adenocarcinoma, a result consistent with the finding that greater than 73% lung cancer patients who are never smokers are diagnosed with adenocarcinoma in the GENEVA dataset.

The association between the IL-6 promoter variants and increased risk for lung cancer was strongly supported by functional studies. Rs1800797 was significantly associated with increased IL-6 mRNA expression in primary HBECs, lymphoblastic cells, and fibroblasts that

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

may at least partially stem from greater gene transcription assessed using luciferase reporter assay. Our cloning strategy recapitulates the physical linear allelic combination for the four common SNPs at this 690-bp promoter region for the two common haplotype alleles studied (A-G-A8T12-C and G-G-A10T11-G) and allows for the detection of potential combinational function of the four individual SNPs on gene transcription, a key factor in detecting the difference in basal expression of IL-6 (Terry et al. 2000). Other studies that compared luciferase activities for two promoter constructs that only differed by one base (rs1800795, G/C) with one allele not naturally existing did not see an increase in basal transcription associated with rs1800795 'C' allele (Cole et al. 2010; Fishman et al. 1998; Kristiansen et al. 2003).

Compelling evidence suggests that IL-6 signaling in the tumor microenvironment is an essential factor promoting tumor cell proliferation, survival, and metastasis (Fisher et al. 2014). Consistent with this supposition, our previous studies support a paracrine dominant mechanism for IL-6 signaling mediated by lung fibroblasts that increases the risk of malignant transformation in human bronchial epithelial cells (Chen et al. 2012). In contrast, inhibition of IL-6 secretion in lung fibroblasts greatly reduced transformation of bronchial epithelial cells exposed chronically to tobacco carcinogens (Chen et al. 2012). The present study observed a strong induction of IL-6 secretion by H_2O_2 or BPDE exposure in fibroblasts. Because the treatment of cells with H_2O_2 and BPDE creates oxidative damage and BPDE-DNA adducts that mimic the damage induced by radon and the tobacco carcinogen benzo(a)pyrene (Narayanan et al. 1997; Tellez et al. 2011), respectively, these findings further implicate the involvement of IL-6 in promoting the development of lung squamous cell carcinoma in uranium miners. Of great importance, assessment of the effect of rs1800797 genotype on the slopes for IL-6 induction by H_2O_2 or BPDE treatments identified greater induction of IL-6 secretion in rs1800797 AG lines

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

than GG lines. Thus, greater levels of basal and carcinogen-induced IL-6 secretion seen for fibroblasts carrying IL-6 variants could lead to a microenvironment that may favor clonal expansion and progression of lung premalignant field defects. This would in turn contribute to the increased risk for lung cancer in populations with exposure to radon and/or cigarette smoke.

Controls were selected from uranium miners with no lung cancer diagnosis during their entire life, thus, our design may be very optimal for a genetic association study because no misclassification for a control becoming a case is possible. However, caution should be taken in the interpretation of the associations between radon exposure, smoking history and risk for lung cancer. WLM was calculated up to cancer diagnosis for cancer cases and for the entire uranium mining occupation for the controls. Smoking history was obtained at the time of sputum collection with the assumption that heavy smokers (≥ 35 packyears) will maintain heavy smoking status during their entire lifetime. It is unknown whether the smoking behavior changes after sputum was collected. Thus, radon and cigarette smoke exposures were not estimated for controls at the point when controls have person-time contributions proportional to cases at cancer diagnosis. A nested case-control study design may be more ideal to address the temporal relationship between radon exposure, smoking history, and risk for lung cancer.

Conclusions

Our findings suggest that sequence variants in the IL-6 promoter modulate gene transcription and responses to environmental carcinogens by lung fibroblasts. In particular, *in vitro* evidence that the rs1800797 variant modulates IL-6 expression supports a role of IL-6 in pathogenic mechanisms associated with squamous cell lung cancer in uranium miners and lung cancer in never smokers.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR. 2002. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nature genetics 30:97-101.

Archer VE. 1988. Lung cancer risks of underground miners: Cohort and case-control studies. The Yale journal of biology and medicine 61:183-193.

Archer VE, Renzetti AD, Doggett RS, Jarvis JQ, Colby TV. 1998. Chronic diffuse interstitial fibrosis of the lung in uranium miners. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine 40:460-474.

Archer VE, Coons T, Saccomanno G, Hong DY. 2004. Latency and the lung cancer epidemic among united states uranium miners. Health physics 87:480-489.

Brenner DR, Boffetta P, Duell EJ, Bickeboller H, Rosenberger A, McCormack V, et al. 2012. Previous lung diseases and lung cancer risk: A pooled analysis from the international lung cancer consortium. American journal of epidemiology 176:573-585.

Chen W, Xu X, Bai L, Padilla MT, Gott KM, Leng S, et al. 2012. Low-dose gamma-irradiation inhibits il-6 secretion from human lung fibroblasts that promotes bronchial epithelial cell transformation by cigarette-smoke carcinogen. Carcinogenesis 33:1368-1374.

Cole SW, Arevalo JM, Takahashi R, Sloan EK, Lutgendorf SK, Sood AK, et al. 2010. Computational identification of gene-social environment interaction at the human il6 locus. Proceedings of the National Academy of Sciences of the United States of America 107:5681-5686.

Dougan M, Li D, Neuberg D, Mihm M, Googe P, Wong KK, et al. 2011. A dual role for the immune response in a mouse model of inflammation-associated lung cancer. The Journal of clinical investigation 121:2436-2446.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Fisher DT, Appenheimer MM, Evans SS. 2014. The two faces of il-6 in the tumor microenvironment. Seminars in immunology 26:38-47.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. 1998. The effect of novel polymorphisms in the interleukin-6 (il-6) gene on il-6 transcription and plasma il-6 levels, and an association with systemic-onset juvenile chronic arthritis. The Journal of clinical investigation 102:1369-1376.

Gao SP, Mark KG, Leslie K, Pao W, Motoi N, Gerald WL, et al. 2007. Mutations in the egfr kinase domain mediate stat3 activation via il-6 production in human lung adenocarcinomas. The Journal of clinical investigation 117:3846-3856.

Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. 2012. An integrated map of genetic variation from 1,092 human genomes. Nature 491:56-65.

Gilliland FD, Hunt WC, Archer VE, Saccomanno G. 2000. Radon progeny exposure and lung cancer risk among non-smoking uranium miners. Health physics 79:365-372.

He JQ, Foreman MG, Shumansky K, Zhang X, Akhabir L, Sin DD, et al. 2009. Associations of il6 polymorphisms with lung function decline and copd. Thorax 64:698-704.

Holm K, Melum E, Franke A, Karlsen TH. 2010. Snpexp - a web tool for calculating and visualizing correlation between hapmap genotypes and gene expression levels. BMC bioinformatics 11:600.

Iyer R, Lehnert BE, Svensson R. 2000. Factors underlying the cell growth-related bystander responses to alpha particles. Cancer research 60:1290-1298.

Kel AE, Gossling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. 2003. Match: A tool for searching transcription factor binding sites in DNA sequences. Nucleic acids research 31:3576-3579.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Krewski D, Lubin JH, Zielinski JM, Alavanja M, Catalan VS, Field RW, et al. 2006. A combined analysis of north american case-control studies of residential radon and lung cancer. Journal of toxicology and environmental health Part A 69:533-597.

Kristiansen OP, Nolsoe RL, Larsen L, Gjesing AM, Johannesen J, Larsen ZM, et al. 2003. Association of a functional 17beta-estradiol sensitive il6-174g/c promoter polymorphism with early-onset type 1 diabetes in females. Human molecular genetics 12:1101-1110.

Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, et al. 2009. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. American journal of human genetics 85:679-691.

Leng S, Stidley CA, Liu Y, Edlund CK, Willink RP, Han Y, et al. 2012. Genetic determinants for promoter hypermethylation in the lungs of smokers: A candidate gene-based study. Cancer research 72:707-715.

Ljungman P, Bellander T, Nyberg F, Lampa E, Jacquemin B, Kolz M, et al. 2009a. DNA variants, plasma levels and variability of interleukin-6 in myocardial infarction survivors: Results from the airgene study. Thrombosis research 124:57-64.

Ljungman P, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R, et al. 2009b. Modification of the interleukin-6 response to air pollution by interleukin-6 and fibrinogen polymorphisms. Environmental health perspectives 117:1373-1379.

Lundin FE, Wagoner JK, Archer VE. 1971. Radon daughter exposure and respiratory cancer quantitative and temporal aspects; report from the epidemiological study of united states uranium miners. Washington:U.S. Public Health Service; available from the National Technical Information Service, Springfield.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Mapel DW, Coultas DB, James DS, Hunt WC, Stidley CA, Gilliland FD. 1997. Ethnic differences in the prevalence of nonmalignant respiratory disease among uranium miners. American journal of public health 87:833-838.

Narayanan PK, Goodwin EH, Lehnert BE. 1997. Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. Cancer research 57:3963-3971.

National Research Council (U.S.). Committee on Health Effects of Exposure to Radon., Samet JM, National Research Council (U.S.). Board on Radiation Effects Research., National Research Council (U.S.). Commission on Life Sciences. 1994. Health effects of exposure to radon : Time for reassessment? Washington, D.C.:National Academy Press.

Ochoa CE, Mirabolfathinejad SG, Ruiz VA, Evans SE, Gagea M, Evans CM, et al. 2011. Interleukin 6, but not t helper 2 cytokines, promotes lung carcinogenesis. Cancer prevention research 4:51-64.

Prise KM, Pinto M, Newman HC, Michael BD. 2001. A review of studies of ionizing radiation-induced double-strand break clustering. Radiation research 156:572-576.

Promega Corporation. 2015. pGL2 Luciferase Reporter Vectors. Available: https://www.promega.com/~/media/files/resources/protocols/technical%20manuals/0/pgl2%20luciferase%20reporter%20vectors%20protocol.pdf [accessed 27 August 2015].

Qi Y, Zhang M, Li H, Frank JA, Dai L, Liu H, et al. 2014. Autophagy inhibition by sustained overproduction of il6 contributes to arsenic carcinogenesis. Cancer research 74:3740-3752.

Rosanna DP, Salvatore C. 2012. Reactive oxygen species, inflammation, and lung diseases. Current pharmaceutical design 18:3889-3900.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Saccomanno G, Auerbach O, Kuschner M, Harley NH, Michels RY, Anderson MW, et al. 1996. A comparison between the localization of lung tumors in uranium miners and in nonminers from 1947 to 1991. Cancer 77:1278-1283.

Samet JM. 1989. Radon and lung cancer. Journal of the National Cancer Institute 81:745-757.

Samet JM, Avila-Tang E, Boffetta P, Hannan LM, Olivo-Marston S, Thun MJ, et al. 2009. Lung cancer in never smokers: Clinical epidemiology and environmental risk factors. Clinical cancer research : an official journal of the American Association for Cancer Research 15:5626-5645.

Schubauer-Berigan MK, Daniels RD, Pinkerton LE. 2009. Radon exposure and mortality among white and american indian uranium miners: An update of the colorado plateau cohort. American journal of epidemiology 169:718-730.

Sethi TK, El-Ghamry MN, Kloecker GH. 2012. Radon and lung cancer. Clinical advances in hematology & oncology : H&O 10:157-164.

Sousa AL, Fava VM, Sampaio LH, Martelli CM, Costa MB, Mira MT, et al. 2012. Genetic and immunological evidence implicates interleukin 6 as a susceptibility gene for leprosy type 2 reaction. The Journal of infectious diseases 205:1417-1424.

Tellez CS, Juri DE, Do K, Bernauer AM, Thomas CL, Damiani LA, et al. 2011. Emt and stem cell-like properties associated with mir-205 and mir-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. Cancer research 71:3087-3097.

Terry CF, Loukaci V, Green FR. 2000. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. The Journal of biological chemistry 275:18138-18144.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, et al. 2008. Association of radiographic emphysema and airflow obstruction with lung cancer. American journal of respiratory and critical care medicine 178:738-744.

Xu Z, Taylor JA. 2009. Snpinfo: Integrating gwas and candidate gene information into functional snp selection for genetic association studies. Nucleic acids research 37:W600-605.

Zakharyan R, Petrek M, Arakelyan A, Mrazek F, Atshemyan S, Boyajyan A. 2012. Interleukin-6 promoter polymorphism and plasma levels in patients with schizophrenia. Tissue antigens 80:136-142.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Table 1. Characteristics of squamous cell carcinoma cases and controls

Variable	Case	Control	P value
N	242	336	
Age at lung cancer diagnosis (yrs, mean ± std)	60.3 ± 10.4	-	
Survival after diagnosis (yrs, median (Q1-Q3))	0.6 (0.1-2.3)	-	
Age at death (yrs, mean ± std)	62.6 ± 10.6	69.2 ± 12.5	2.8×10^{-10} [a]
Gender (male %)	100	100	
Ethnicity (NHW %)	100	100	
WLM (kWLMs [b], median (Q1-Q3))	1.0 (0.5-2.1)	0.8 (0.4-1.5)	0.001 [c]
< 0.895 (%)	41.1	54.0	0.055 [d]
≥ 0.895 (%)	48.6	45.4	
Missing (%)	10.4	0.6	
Mid-induction latency (yrs, median (Q1-Q3)) [e]	19 (12.0-26.5)		

[a] Student t test.
[b] Abbreviation kilo. Twenty seven miners have missing cumulative WLM estimate. WLM, a time-integrated measure, was calculated as the product of time in working months (1 month = 170 h) and working levels to estimate the cumulative exposure to radon daughter radiation for each miner.
[c] Wilcoxon rank sum test.
[d] χ^2 test for difference between cases and controls. Missing group was not included in this test.
[e] Squamous cell carcinoma cases (n=162) with complete information for latency and genotypes. Mid-induction latency defined as time from midpoint of the period of substantial exposure (an annual accumulative dose of ≥50.6 mSv) to squamous cell carcinoma diagnosis (Archer et al. 2004).

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Table 2. Association between IL-6 promoter SNPs and squamous cell carcinoma in miners (odds ratios based on adjusted logistic regression models of 242 cases and 336 controls) and hazard ratios for the time from the onset of high exposure to diagnosis among 162 cases

SNP	Allele [a]	Case-control study of squamous cell carcinoma [b]			Mid-induction latency [c]	
		Case [d]	Control [d]	OR (95%CI) [e]	Average (years) [f]	Hazard Ratio [e]
rs12700386	C/G	0.66/0.29/0.05	0.65/0.31/0.04	0.94 (0.69, 1.30)	19.3/20.1/18.4	0.81 (0.59, 1.11)
rs2069827	G/T	0.84/0.15/0.01	0.83/0.14/0.04	0.84 (0.56, 1.26)	19.5/19.9/13.8	1.30 (0.86, 1.95)
rs1800797	G/A	0.30/0.50/0.21	0.39/0.45/0.16	1.36 (1.05, 1.75)	20.7/19.1/18.8	1.57 (1.22, 2.01)
rs2069840	C/G	0.45/0.45/0.10	0.44/0.37/0.19	0.77 (0.60, 1.01)	20.1/19.3/17.5	0.95 (0.71, 1.28)

[a] All forward strand RefSNP alleles. Alleles underlined were minor allele and test allele in the logistic regression.
[b] Adjustment for cumulative WLM estimate, packyears and age at sputum collection was included in logistic regression models. Analysis was conducted in 242 lung squamous cell carcinoma cases and 336 controls.
[c] Adjustment for underground mining history and smoking history surveyed at squamous cell carcinoma diagnosis was included in Cox regression models. Analysis was conducted in 162 cases due to unavailability of the complete record of the occupational history for underground uranium mining.
[d] Forward slashes separated the frequencies of wild homozygotes, heterozygotes, and variant homozygotes for each individual SNPs.
[e] OR or hazard ratio were calculated for the association between genotype of each IL-6 variant and risk for squamous cell carcinoma or latency, respectively. Each variant was coded as 0, 1, and 2 for wild homozygote, heterozygote, and variant homozygote.
[f] Forward slashes separated the average mid-induction latency for subjects carrying wild homozygotes, heterozygotes, and variant homozygotes for each individual SNPs. Mid-induction latency defined as time from midpoint of the period of substantial exposure (an annual accumulative dose of ≥50.6 mSv) to squamous cell carcinoma diagnosis (Archer et al. 2004).

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Table 3. Association between rs1800797 and risk for lung cancer in the GENEVA dataset

Variable	Control (n) [a]	Case (n) [a]	OR (95% CI)	p-Value
Overall	0.67 ± 0.67 (2725)	0.70 ± 0.67 (2522)	1.10 (1.01 – 1.20)	0.045 [b]
Smoking status [c]				
Never smokers	0.60 ± 0.63 (633)	0.70 ± 0.64 (138)	1.41 (1.05 – 1.91)	0.024 [d]
Former smokers	0.67 ± 0.68 (1125)	0.72 ± 0.69 (1204)	1.08 (0.95 – 1.24)	0.24 [d]
Current smokers	0.70 ± 0.68 (967)	0.67 ± 0.66 (1180)	1.06 (0.92 – 1.22)	0.43 [d]
Histology				
Adenocarcinoma	0.67 ± 0.67 (2725)	0.72 ± 0.69 (986)	1.16 (1.04 – 1.31)	0.0091 [d]
Squamous cell carcinoma	0.67 ± 0.67 (2725)	0.69 ± 0.67 (582)	1.08 (0.93 – 1.25)	0.33 [d]
Small cell	0.67 ± 0.67 (2725)	0.62 ± 0.65 (256)	0.90 (0.73 – 1.11)	0.32 [d]
Others	0.67 ± 0.67 (2725)	0.70 ± 0.67 (698)	1.04 (0.91 – 1.19)	0.59 [d]

[a] A allele dosage for rs1800797 presented as mean ± SD

[b] Adjustment for age, sex, smoking status, packyears, and cohort was included in logistic regression models.

[c] The interaction terms between never versus current smokers and rs1800797 and between former versus current smokers and rs1800797 were included together in the logistic regression with statistical significance identified for both interaction terms (p-Values=0.050 and 0.029, respectively).

[d] Adjustment for age, sex, packyears, and cohort was included in logistic regression models.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Table 4. Association between rs1800797 and IL-6 expression in primary human BECs and lymphoblastic cell lines

Tissues [a]	Rs1800797	n	IL-6 expression (mean ± SD)	p-Value
Human BECs				0.009[b]
	GG	42	0.0016 ± 0.0019	
	GA	32	0.0031 ± 0.0036	
	AA	11	0.0041 ± 0.0038	
Lymphoblastic cell lines				0.034[c]
	GG	18	6.18 ± 0.33	
	GA	43	6.19 ± 0.33	
	AA	18	6.36 ± 0.37	

[a] Primary human BEC cultures (n = 85) were established from cells obtained by bronchoscopy from current or former smokers seen at pulmonary clinic of University of New Mexico Hospital. BEC cultures established from unaffected sites were used for this experiment for patients with subsequent lung cancer diagnosis. EBV-transformed lymphoblastoid cell lines (n = 79) from CEU families were established by the HapMap project.

[b] Generalized linear model with adjustment for lung cancer diagnosis status. Rs1800797 genotype was coded as 0, 1, and 2 for GG, GA, and AA genotype. IL-6 expression was expressed as relative quantification with β-actin as the endogenous control.

[c] Likelihood ratio test implemented in Merlin was used to evaluate the association between rs1800797 and IL-6 expression (GI_10834983-S). The family structure and gender were included in the models for covariate adjustment.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

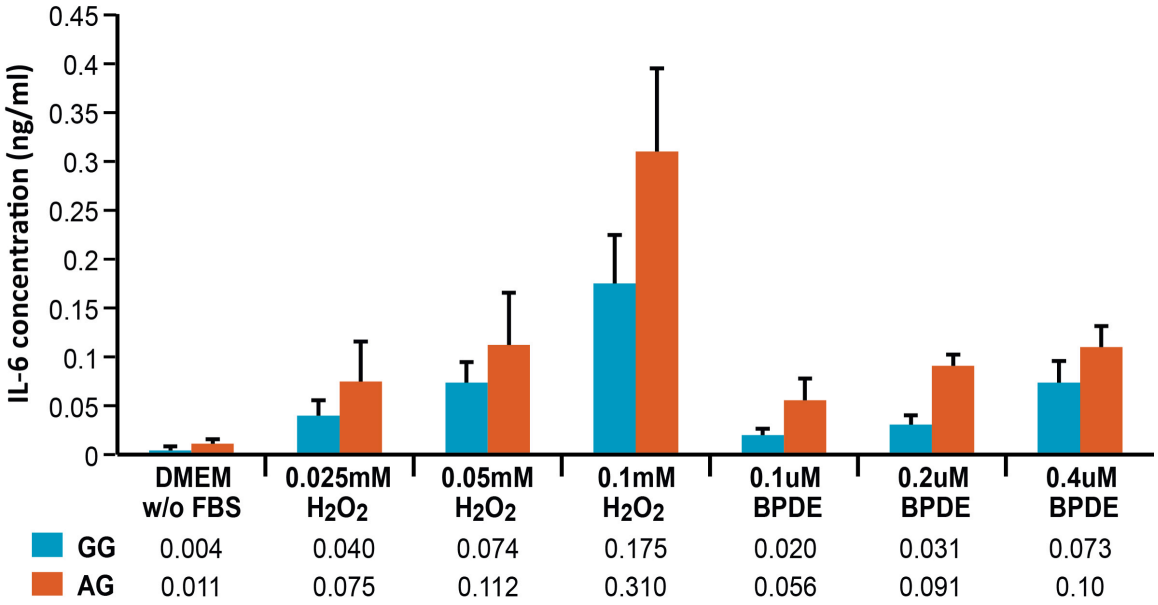
Figure legends

Figure 1. Promoter SNPs affect IL-6 secretion in fibroblast cells treated with H_2O_2 and BPDE. Six fibroblast cell lines, three wild homozygotes (GG) and three heterozygotes (AG) for rs1800797 were treated with H_2O_2 and BPDE. The height of the bar is the average concentration of IL-6 in culture medium that is also expressed as the number under the figure. The error bar is the standard deviation from three independent experiments. Generalized linear model was used to assess the effect of H_2O_2 or BPDE treatment and rs1800797 genotype on IL-6 concentration detected in the medium. A strong IL-6 induction was identified for both carcinogens (p-Values < 0.0001). Moreover, the slope for the induction of IL-6 secretion by H_2O_2 or BPDE treatment is 74% and 39% greater in AG lines than GG lines (p-Values = 0.017 for H_2O_2 and 0.13 for BPDE).

Figure 2. IL-6 promoter activity by haplotype alleles. Luciferase reporter construct containing haplotype allele A-G-A8T12-C (Risk HAP) that carries variant alleles for rs1800797 (A) and rs1800795 (C) has significantly higher reporter activity than seen for G-G-A10T11-G (Reference HAP) in HEK293 (p-Value = 3.42×10^{-6}), HFL1 (p-Value = 1.86×10^{-5}), and HBEC2 (p-Value = 6.2×10^{-4}). The height of the bar is the average luciferase activity standardized by the levels seen for G-G-A10T11-G set to one. The error bar is the standard deviation.

Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Figure 1.



Environ Health Perspect DOI: 10.1289/ehp.1409437
Advance Publication: Not Copyedited

Figure 2.